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Customer No.: 07278

PATENT TRADEMARK OFFICE

Docket No: 0632/0D916

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Hsien-Jue CHU

Serial No.: 09/007,385 Art Unit: 1647

Confirmation No.:

Filed: January 15, 1998 Examiner: S. Turner

For: STREPTOCOCCUS EQUI COMPOSITIONS AND METHODS OF USE

DECLARATION UNDER 37 C.F.R. §1.132 OF HSIEN-JUE CHU

Considered 12-6-01 ST

Hon. Commissioner of
Patents and Trademarks
Washington, DC 20231

June 29, 2001

Sir:

10-1-01 RCE Not Considered 7-26-01 ST

I, Hsien-Jue Chu, declare that:

1. I am a citizen of the United States of America and reside at 1506 13th Avenue North, Forth Dodge, Iowa 50501.

2 I received a Ph.D. degree in Microbiology in 1984 from the University of California, Davis.

3. I am currently a Senior Vice President, Research & Development at Fort Dodge Laboratories, Fort Dodge, Iowa (FDL) and a specialist in Vaccine & Drug Research & Development. I have been employed by (FDL) since October 26, 1987.

4. I am the named inventor of the above-identified U.S. patent application.

5. The following studies were performed to demonstrate the efficacy of the composition of the present invention against a virulent *S. equi* challenge.

A. MATERIALS AND METHODS

i. Test Animals

Sixty-three *S. equi* negative, clinically healthy horses were utilized in this study.

ii. Vaccine Composition and Vaccination Schedule

The preparation containing vaccine organisms used in this study was prepared as described in Example 1 of the above-captioned application. The lyophilized *S. equi* preparation was reconstituted with deionized water containing 2.5 mg/ml of saponin. Adjustments to obtain the target viable count were made as described in Example 1. The vaccine was stored at 2-7°C. The commercial vaccine was used according to the manufacturer's instructions.

iii. Experimental Design

Table 1 below shows the format of the study. The vaccine composition of the claimed invention was administered intranasally to horses in Group 1 using a syringe connected to a flexible tubing five inches in length. The commercial vaccine containing adjuvanted (Carbopol) *S. equi* enzyme extract (Group 2) was administered

intramuscularly according to manufacturer's instructions. The horses were given two vaccinations administered three weeks apart. The control horses were not vaccinated.

Table 1

Group	No. of Horses	First Dose	Second Dose
1	11	$1x10^8$ CFU/dose	$2x10^7$ CFU/dose
2	11	Commercial (Bayer) vaccine	Commercial (Bayer) vaccine
3	11	Control - No Vaccine	No Vaccine

iv. Challenge

Twenty-one days after the second vaccination, each of the 22 vaccinated and 11 control horses were challenged intranasally with one ml (@ $5.2x10^7$ CFU/ml) virulent *S. equi* organism (isolate CF32), which was prepared and stored as described in Example 1 of the above-captioned application.

v. Observation Procedure

The animals were observed daily from -1 days to 0 days post challenge (DPC) to establish a baseline and 1 to 21 DPC (excluding 18 and 20 DPC) for various clinical signs. Animals were further observed on 23, 26, 28, 33 and 35 DPC.

vi. Clinical Scoring System

Clinical signs of *S. equi* infection in horses were measured as shown in Table 2 and an average clinical score was determined for each group of horses.

Table 2

Clinical Symptom	Score
Coughing	1 Point/Day
Nasal discharge (1) Serous (2) Mucopurulent	 1 Point/Day 2 Points/Day
Ocular discharge	1 Point/Day
Depression	1 Point/Day
Pyrexia	1 point for temperatures between 103.0 and 104.0°F; 2 points for temperatures between 104.0 and 105.0°F
Labored breathing	2 Points/Day
Enlargement of lymph nodes (1) Head and neck areas (2) Disseminated*	 2 Points/Day 3 Points/Day
Abscesses of lymph nodes (1) Head and neck areas (2) Disseminated	 25 Points/One Time Score 40 Points/One Time Score
Death	(150 Points/One Time Score)

* Other than submandibular and pharyngeal lymph nodes.

vii. Statistical Analysis

The level of significance for each statistical analysis was set at $p < 0.05$. All analysis was completed on an IBM computer using SAS software. Mortality was compared using the Fishers Exact test. Total clinical score was compared using the Mann-Whitney U test. Swollen lymph node incidence and incidence of lymph node rupture were compared using Fishers Exact test.

B. RESULTS

i. Clinical Observations

Ruptured abscesses % occurrence

Group 1	36
Group 2	81
Group 3	73

ii. Average clinical score

Group 1	23.6
Group 2	53.9
Group 3	67.4

C. DISCUSSION

A reduction in the occurrence of ruptured abscesses of 51% was observed in the horses administered the composition of the invention compared to the control horses and a reduction of 56% was observed compared to the horses administered the commercial intramuscular vaccine.

A reduction of 65% in the average clinical score was observed in the horses administered the composition of the invention compared to the control horses. A reduction of 56% compared to the horses administered the commercial intramuscular vaccine was observed.

The results demonstrate a significant reduction of disease incidence in the group administered the claimed intranasal *S. equi*/saponin vaccine in comparison to the control or the commercial intramuscular vaccine without saponin.

6. The *S. equi*/saponin composition of the present invention provided immunity and protection for intranasally vaccinated horses against strangles. The composition of the invention provided enhanced protection over the commercially available adjuvanted *S. equi* extract composition for intramuscular vaccination.

7. I declare further that statements made in this Declaration are of my own knowledge and are true and that all statements made on information and belief are believed to be true and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 28 June 2001

Hsien-Jue Chu

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